In re application of
Baird et al
Application No.: 09/372,474

Atty. Dkt. No. 025098-0701

Amendments to the Claims/Listing of Claims

Please cancel claims 46-49 without prejudice. This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Previously presented) A method for designing a specific polyamide

$$X_1X_2 \ldots X_m\text{-}\gamma\text{-}X_{(m+1)} \ldots X_{(2m-1)}X_{2m}\text{-}R_1$$

wherein

X_1, X_2, X_m, $X_{(m+1)}$, $X_{(2m-1)}$, and X_{2m} are carboxamide residues forming carboxamide binding pairs X_1/X_{2m}, $X_2/X_{(2m-1)}$, $X_m/X_{(m+1)}$;

γ is γ-aminobutyric acid or 2,4 diaminobutyric acid, and

R_1 is $-NH(CH_2)_{0\text{-}100}NR_2R_3$, $-NH(CH_2)_{0\text{-}12}CONH(CH_2)_{0\text{-}100}NR_2R_3$, or $-NHR_2$, where R_2 and R_3 are independently selected from the group consisting of H, Cl, NO, N-acetyl, benzyl, $C_{1\text{-}100}$ alkyl, $C_{1\text{-}100}$ alkylamine, $C_{1\text{-}100}$ alkyldiamine, $C_{1\text{-}100}$ alkylcarboxylate, $C_{1\text{-}100}$ alkenyl, a $C_{1\text{-}100}$ alkynyl, and $C_{1\text{-}100}$ alkyl-L, where L is selected from the group consisting of arylboronic acids, biotins, polyhistidines comprised from about 2 to 8 amino acids, haptens, solid phase supports, oligodeoxynucleotides, N-ethylnitrosourea, fluorescein, bromoacetamide, iodoacetamide, DL-α-lipoic acid, acridine, captothesin, pyrene, mitomycin, texas red, anthracene, anthrinilic acid, avidin, DAPI, and oligodeoxynucleotide, isosulfan blue, malachite green, psoralen, ethyl red, 4-(psoraen-8-yloxy)-butyrate, tartaric acid, and (+)-α-tocopheral, suitable for use as a DNA-binding ligand that is selective for identified target DNA-sequences 5'-$WN_1N_2 \ldots N_mW$-3' where m is an integer having a value from 3 to 6, the method comprising:

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(a) identifying a target sequence of double stranded DNA having the form 5'-$WN_1N_2 \ldots N_mW$-3', $N_1N_2 \ldots N_m$ being the sequence to be bound by carboxamide residues, wherein each N is independently chosen from the group A, G, C, and T, each W is independently chosen from the group A and T, and m is an integer having a value from 3 to 6;

(b) representing the identified sequence as 5'-W*ab* ... *x*W-3', wherein *a* is a first nucleotide to be bound by the X_1 carboxamide residue, *b* is a second nucleotide to be bound by the X_2 carboxamide residue, and *x* is the corresponding nucleotide to be bound by the X_m carboxamide residue;

(c) defining *a* as A, G, C, or T to correspond to the first nucleotide to be bound by a carboxamide residue in the identified sequence;

(d) selecting Im as the X_1 carboxamide residue and Py as the X_{2m} carboxamide residue if a = G;

(e) selecting Py as the X_1 carboxamide residue and Im as the X_{2m} carboxamide residue if *a* = C;

(f) selecting Hp as the X_1 carboxamide residue and Py as the X_{2m} carboxamide residue if *a* = T;

(g) selecting Py as the X_1 carboxamide residue and Hp as the X_{2m} carboxamide residue if *a* = A; and

(h) repeating steps c – g for *b* through *x* until all carboxamide residues are selected;

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wherein Im is N-methylimidazole, Hp is 3-hydroxy-N-methylpyrrole , Py is N-methylpyrrole, A is adenine, G is guanine, C is cytosine, and T is thymine; and

synthesizing the polyamide.

2. (Cancelled)

3. (Previously presented) The method of claim 1 further comprising the step of determining if the binding affinity of the polyamide to the identified target sequence is subnanomolar.

4. (Previously presented) The method of claim 1 further comprising the step of determining if the polyamide exhibits a binding affinity that is at least ten-fold higher for said identified target sequence compared to a non-target DNA sequence.

5. (Previously presented) The method of claim 1 further comprising the step of replacing at least one pyrrole residue with a β-alanine residue.

6-41. (Cancelled)

42. (Previously presented) The method of claim 1 wherein the identified target DNA sequence is a regulatory sequence.

43. (Previously presented) The method of claim 1 wherein the identified target DNA sequence is a promoter sequence.

44. (Previously presented) The method of claim 1 wherein the identified target DNA sequence is a coding sequence.

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45. (Previously presented) The method of claim 1 wherein the identified target DNA sequence is a non-coding sequence.

46. – 49. (Cancelled)

50. (Previously presented) A method for designing a specific polyamide

$$X_1X_2 \ldots X_m\text{-}\gamma\text{-}X_{(m+1)} \ldots X_{(2m-1)}X_{2m}\text{-}R_1$$

wherein

X_1, X_2, X_m, $X_{(m+1)}$, $X_{(2m-1)}$, and X_{2m} are carboxamide residues forming carboxamide binding pairs X_1/X_{2m}, $X_2/X_{(2m-1)}$, $X_m/X_{(m+1)}$, and wherein one carboxamide binding pair is substituted with a β/β, wherein β is β-alanine;

γ is γ-aminobutyric acid or 2,4 diaminobutyric acid, and

R_1 is $-NH(CH_2)_{0\text{-}100}NR_2R_3$, $-NH(CH_2)_{0\text{-}12}CONH(CH_2)_{0\text{-}100}NR_2R_3$, or $-NHR_2$, where R_2 and R_3 are independently selected from the group consisting of H, Cl, NO, N-acetyl, benzyl, $C_{1\text{-}100}$ alkyl, $C_{1\text{-}100}$ alkylamine, $C_{1\text{-}100}$ alkyldiamine, $C_{1\text{-}100}$ alkylcarboxylate, $C_{1\text{-}100}$ alkenyl, a $C_{1\text{-}100}$ alkynyl, and $C_{1\text{-}100}$ alkyl-L, where L is selected from the group consisting of arylboronic acids, biotins, polyhistidines comprised from about 2 to 8 amino acids, haptens, solid phase supports, oligodeoxynucleotides, N-ethylnitrosourea, fluorescein, bromoacetamide, iodoacetamide, DL-α-lipoic acid, acridine, captothesin, pyrene, mitomycin, texas red, anthracene, anthrinilic acid, avidin, DAPI, and oligodeoxynucleotide, isosulfan blue, malachite green, psoralen, ethyl red, 4-(psoraen-8-yloxy)-butyrate, tartaric acid, and (+)-α-tocopheral, suitable for use as a DNA-binding

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ligand that is selective for identified target DNA-sequences 5'-$WN_1N_2 \ldots N_mW$-3' where **m is** an integer having a value from 3 to 6, the method comprising:

(a) identifying a target sequence of double stranded DNA having the form 5'-$WN_1N_2 \ldots N_mW$-3', $N_1N_2 \ldots N_m$ being the sequence to be bound by carboxamide residues, wherein each N is independently chosen from the group A, G, C, and T, each W is independently chosen from the group A and T, and m is an integer having a value from 3 to 6;

(b) representing the identified sequence as 5'-W*ab* ... *x*W-3', wherein *a* is a first nucleotide to be bound by the X_1 carboxamide residue, *b* is a second nucleotide to be bound by the X_2 carboxamide residue, and *x* is the corresponding nucleotide to be bound by the X_m carboxamide residue;

(c) defining *a* as A, G, C, or T to correspond to the first nucleotide to be bound by a carboxamide residue in the identified sequence;

(d) selecting Im as the X_1 carboxamide residue and Py as the X_{2m} carboxamide residue if a = G;

(e) selecting Py as the X_1 carboxamide residue and Im as the X_{2m} carboxamide residue if *a* = C;

(f) selecting Hp as the X_1 carboxamide residue and Py as the X_{2m} carboxamide residue if *a* = T;

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(g) selecting Py as the X_1 carboxamide residue and Hp as the X_{2m} carboxamide residue if a = A; and

(h) repeating steps c – g for b through x until all carboxamide residues are selected;

wherein Im is N-methylimidazole, Hp is 3-hydroxy-N-methylpyrrole , Py is N-methylpyrrole, A is adenine, G is guanine, C is cytosine, and T is thymine; and

synthesizing the polyamide.

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